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Alterations of homocysteine serum levels during alcohol withdrawal are influenced by riboflavin – results from the German Investigation on Neurobiology in Alcoholism (GINA)

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Abstract

Background: Various studies have shown that plasma homocysteine serum levels are elevated in actively drinking alcohol dependent patients a during alcohol withdrawal, while rapidly declining during abstinence. Hyperhomocysteinaemia has been associated not only with blood alcohol concentration, but also with deficiency of different B-vitamins, particularly folate, pyridoxine and cobalamin.

Methods: Our study included 168 inpatients (110 men, 58 women) after admission for detoxification treatment. Blood alcohol concentration, folate, cobalamin, pyridoxine, thiamine and riboflavin were obtained on admission (day 1). Homocysteine was assessed on days 1, 7 and 11.

Results: Homocysteine levels significantly declined during withdrawal. General linear models and linear regression analysis showed an influence of blood alcohol concentration, folate and riboflavin on the homocysteine levels on admission as well as on homocysteine changes occurring during alcohol withdrawal. No significant influence was found for thiamine, cobalamin and pyridoxine.

Discussion: These findings show that not only blood alcohol concentration and plasma folate levels, but also plasma levels of riboflavin influence homocysteine plasma levels in alcohol dependent patients.

Keywords: homocysteine, alcohol dependence, alcoholism, folate, withdrawal, riboflavine, MTHFR, B-vitamins

Introduction

Homocysteine (HCY) metabolism is known to be fundamentally altered during alcohol intoxication and withdrawal (Bleich et al., 2005; Bleich et al., 2004; Bleich et al., 2000b). This has been shown for alcohol dependent patients, but also – to a lesser extent – for social drinkers (Bleich et al., 2001). HCY levels found in alcohol dependent patients tend to be much higher than the common cut-off levels for HCY, defined in a study of Ubbink and colleagues as 11.7µmol/l (Ubbink et al., 1995) or in a different investigation as 11.4µmol/l for male and 10.4µmol/l for female subjects (Selhub et al., 1999).

The amino acid HCY is known to increase glutamatergic neurotransmission via activation of the N-Methyl-D-Aspartate (NMDA)-receptor of the glutamatergic system (Bleich et al., 2004; Bleich and Hillemacher, 2009). The glutamatergic system is of crucial importance not only for withdrawal symptoms but also for the individual relapse risk and is targeted by, e.g., the anti-craving compound acamprosate (Hillemacher et al., 2007b; Umhau et al., 2010). Elevated homocysteine levels lead to neurodegeneration and have been associated – inter alia – with vascular diseases and brain atrophy (Sachdev, 2004; Wilhelm et al., 2008). During alcohol withdrawal neurotransmission via the (up-regulated) NMDA-receptors is not longer blocked by ethanol, resulting in an extensive overstimulation by HCY-associated NMDA-receptor agonism. This extensive glutamatergic reaction has been assumed to be the neurobiological background of the association between elevated HCY levels and the higher risk of alcohol withdrawal seizures (Bayerlein et al., 2005; Bleich et al., 2006; Bleich et al., 2000a; Hillemacher et al., 2007a). Furthermore, elevated HCY levels have been found to correlate with short-term cognition deficits during alcohol withdrawal (Wilhelm et al., 2006) as well as long-term cerebral atrophy (Bleich et al., 2003).

This increase in HCY levels in alcohol dependent patients is based on direct effects of ethanol (Bleich et al., 2005; Bleich et al., 2000b) as well as an alcohol-associated deficiency of folate

(vitamin B9), cobalamine (vitamin B12), pyridoxine (vitamin B6) (Bleich et al., 2004; Chen et al.; Cravo and Camilo, 2000).

The present study was performed to validate previous findings investigating alterations of HCY serum levels during alcohol withdrawal in a large population of alcohol dependent patients and to study the role of the B-vitamins involved in homocysteine metabolism in the pathogenesis of alcohol-associated hyperhomocysteinemia.

Subjects and methods

The present study was part of the German Investigation on Neurobiology in Alcoholism (GINA). All patients were recruited from the Department of Addiction and Psychotherapy of the hospital "Rheinische Kliniken" in Bonn, Germany. Written informed consent was obtained from all 363 patients (251 men, 112 women). In a subsample of serial patients, we performed a prospective approach, obtaining data on day 1, day 7 and day 11 after admission for detoxification treatment. This subsample was used in the present analysis, consisting of 168 patients (110 men, 58 women). All procedures were approved by the local ethics committee of the University of Bonn, Germany, and were in accordance with the Helsinki Declaration of 1975, as revised in 1983. All participants suffered from alcohol dependency according to ICD-10 and were included in the study on admission for alcohol detoxification. Patients were mainly detoxified using clomethiazole following a symptom-triggered regime using the Banger-Score (Banger et al., 1992). If, for clinical reasons, clomethiazole could not be used, benzodiazepines were administered. Patients suffering from dependence of other substances except alcohol and nicotine were excluded from the study.

Laboratory measurements:

Fasting blood samples were obtained on day 1 (admission), day 7 and day 11 of detoxification treatment. Blood samples were centrifuged and consecutive serum and lithium heparin plasma

samples were stored at -80ºC directly after collection. HCY was assessed at all three time points while vitamin serum levels were obtained at admission.

Plasma total HCY concentrations were measured by means of particle-enhanced immunonephelometry with a Dimension Vista™ system according to the manufacturer's instructions (Siemens Healthcare Diagnostics, Eschborn, Germany). The reference interval given by Siemens Healthcare Diagnostics was 4.9-15.0 µmol/l. Serum vitamin B12 and folate concentrations were measured by means of a homogenous, competitive chemiluminescent immunoassay based on the LOCI™ technology with a Dimension Vista™ system (Siemens Healthcare Diagnostics, Eschborn, Germany). Reference intervals given by Siemens Healthcare Diagnostics were 3.1-17.5 ng/ml for folate and 254–1320 pg/ml for cobalamin.

Serum alcohol concentration was measured by means of an enzymatic test (alcohol dehydrogenase method) with a Dimension Vista™ system (Siemens Healthcare Diagnostics).

Blood concentrations of thiamine, riboflavin and pyridoxine were analyzed using commercially available HPLC assays (Chromsystems Instruments & Chemicals GmbH, Munich, Germany) on a HPLC system with a fluorescence detector (Agilent.Series 1200, Agilent, Waldbronn, Germany). Reference intervals given by Chromsystems were 66.5 – 200 nnmol/l for thiamine, 174 – 471 nmol/l for riboflavin and 8.7 – 27.3 µg/l for pyridoxine.

Statistical assessment:

Results are presented as mean plus standard deviation (SD) or standard error (SE). Variables were normally distributed as tested using the Kolmogorov-Smirnov test. Linear regression models and general linear models for repeat measurements were used to test the influence of different variables on HCY levels. Significance level (p-value) was set below 0.05.

Data were analyzed using IBM SPSS Statistics 19.0 and Graph Pad Prism^{TM} 5.0 (Graph Pad Software Inc., San Diego, CA).

Results

Demographic characteristics of the study population are given in *Table 1*. Very few patients suffered from a definitive B-vitamine deficiency (two patient presented folate deficiency, one patient thiamine deficiency, yet none of the other included B-vitamins).

Statistical analysis using general linear models for repeat measurements showed that homocysteine levels decline rapidly during alcohol withdrawal ($F=45.28$, $p<0.001$), with significant differences between all time points (day 1/day7: $p<0.001$; day1/day11: $p<0.001$; day7/day11: $p<0.001$; *Figure 1*). A multivariate linear regression model showed that HCY serum levels on day 1 of alcohol withdrawal are significantly influenced by BAC on admission ($B=1.60$, $T=2.30$, $p=0.022$), folate ($B=-0.75$, $T=-5.47$, $p<0.001$) and riboflavin ($B=-0.06$, $T=-3.44$, $p=0.001$), but not by thiamine, pyridoxine or cobalamin serum levels. Correlation analysis revealed no significant association between folate and riboflavin levels ($r=0.119$, $p=0.121$). To test which factors influence the changes of HCY levels over time (day 1, day 7, day 11), we performed a general linear model computing BAC and vitamin levels as covariates. We found a significant interaction between HCY changes over time and BAC on admission ($F=4.80$, $p=0.009$), folate serum levels on admission ($F=7.02$, $p=0.001$) and riboflavin serum levels on admission ($F=3.228$, $p=0.042$), but not with other tested variables (thiamine, pyridoxine or cobalamin). Including the interaction between folate and riboflavin and omitting thiamine, pyridoxine and cobalamin results did not relevantly differ regarding the influence of thiamine, riboflavin and BAC on changes of HCY over time without showing a significant effect of the interaction (folate/riboflavin: $F=1.56$, $p=0.214$).

Comparing the subgroups of patients with high (HCY>15.35µmol/l, N=84) and low (HCY<=15.36µmol/l, N=84) HCY serum levels at admission, t-test for independent samples revealed that the high HCY group also showed significantly elevated HCY levels on day 7 ($T=6.4$, $p<0.001$) and elevated HCY levels on day 11 ($T=6.0$, $p<0.0001$) as well as reduced

folate (T=-6.3, p<0.001), riboflavin (T=-2.7, p=0.008) and pyridoxine (T=-2.1, p=0.039) serum levels at admission.

Discussion

The present investigation confirms previous observations that reported elevated HCY levels in patients with alcohol dependence undergoing detoxification treatment. As shown previously, on the time of admission the extent of the HCY serum levels depends significantly on BAC (Bleich et al., 2005; Bleich et al., 2000b). However, apart from BAC, serum levels of riboflavin and folate also showed a significant influence on HCY serum levels on admission. The same result was found when general linear model analysis was performed, analyzing the effect of BAC and B-vitamins on the changes of HCY during withdrawal. Interestingly, cobalamin and pyridoxine showed no significant influence on HCY. However, riboflavin, which, to our knowledge, has not been studied in the context of alcohol-associated hyperhomocysteinemia before, showed a significant impact on HCY levels, which did not depend on its interaction with folate. Riboflavin is a precursor of various flavin-coenzymes, particularly of the flavin adenine dinucleotide (FAD), which is a coenzyme of methylenetetrahydrofolate reductase MTHFR (Moat et al., 2003). Substituted by FAD MTHFR catalyses 5,10-methylenetetrafolate to 5-methylenetetrafolate, which is of importance as a donor of methyl groups for remethylation of homocysteine (Moat et al., 2003). Accordingly, MTHFR activity is reduced in ribloflavin-deficient rats (Bates and Fuller, 1986).

Overall, our results show that apart from individual BAC folate and riboflavin plasma levels on admission contribute to HCY elevation in alcohol dependent patients. This effect was not only shown at the beginning of in-patient treatment but also regarding the decline of HCY during the withdrawal period. Noteworthy, only a negligible number of patients suffered from a definite B-vitamin deficiency. Thus, the present findings support the hypothesis that a

supplementation of folate and riboflavin may be useful in alcohol dependent patients during active drinking as well as detoxification and that currently used reference values for B-vitamins may need to be adjusted for alcohol dependent patients.

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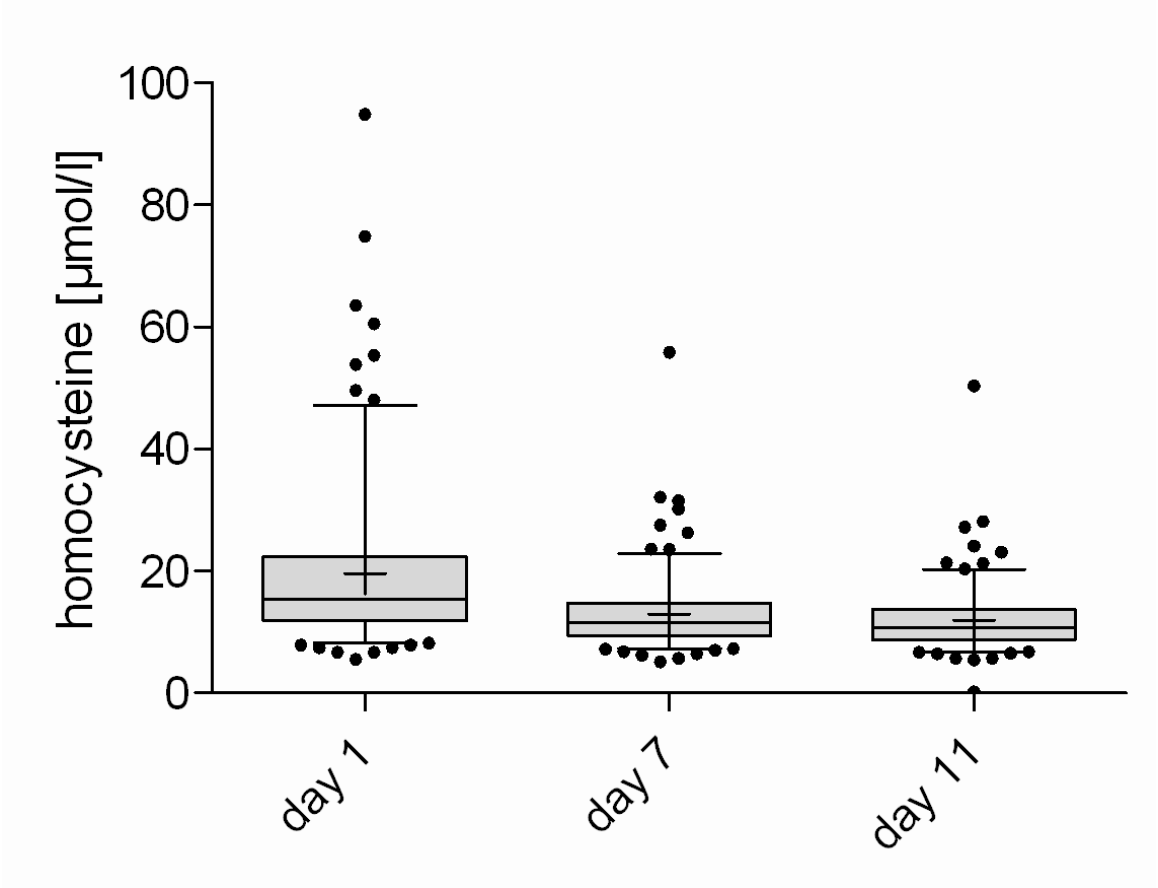
Table 1: Demographic characteristics of the study population

	mean	SD	median	range
age (years)	48.1	8.7	48.0	21-66
BAC (per mille)	1.46	1.1	1.43	0-4.2
HCY day 1 (µmol/l)	19.6	12,9	15.3	5.5-94.9
HCY day 7 (µmol/l)	12.9	5.9	11.6	5.1-55.9
HCY day 11 (µmol/l)	11.9	5.1	10.7	0.2-50.4
folate (ng/ml)	9.8	5.7	9.1	1.5-20.0
riboflavin (nmol/l)	295.1	51.0	288.6	192.7-480.1
thiamine (nmol/l)	164.4	47.1	160.9	16.8-338.6
pyridoxine (µg/l)	31.4	48.9	25.1	10.6-522.6
cobalamin (pg/ml)	705.7	293.8	650.0	228.0-1500.0
Sex	male: N=110		female: N=58	

Legend table 1:

SD: standard deviation, BAC: blood alcohol concentration at admission; HCY: homocysteine serum levels, measured at day 1, day 7 or day 11 after admission for in-patients detoxification treatment. Vitamin levels were measured at admission.

Figure 1: Decrease of homocysteine serum levels during alcohol withdrawal



Legend Figure 1

Box plot showing changes of homocysteine plasma levels during withdrawal. Box represents 25-75 percentile, whiskers represent 5-95 percentile. Bar represents median, cross represents mean. Homocysteine plasma levels decreased significantly during the course of withdrawal. Statistical details are summarized in the results section.